In the Specification:

Please delete the earlier filed Sequence Listing and replace with the new Sequence Listing attached herewith.

Please amend as follows. Text added to the paragraph is presented in underlined format, while text to be deleted is presented in strike-through format.

Please replace paragraph [0004] with the following paragraph:

[0004] Over 80% of patients with ML IV are of Ashkenazi-Jewish descent. The estimated carrier rate of ML IV is 1:100 with an incidence of about 1:40,000. Two mutations in the MCOLN1 gene account for 95% of the Ashkenazi Jewish ML IV alleles. An A>G transition (IVS 3-2 A>G), which causes a splice site mutation in the acceptor site of intron 3 is found in 72% of Ashkenazi ML IV alleles. A 6.4 kb deletion that includes exons 1 to 7 of the MCOLN1 gene (GenBank accession no. AF287270**; SEQ ID NO: 8**) is found in 23% of Ashkenazi ML IV alleles. See e.g., Bach (2001) *Molecular Genetics and Metabolism* 73: 197-203; Bargal et al., (2001) *Human Mutation* 17:397-402; Bargal et al., (2000) *Nature Genetics* 26: 118-121; Bassi et al., (2000) *Am. J. Genet.* 67:1110-1120; and Sun et al., (2000) *Human Molecular Genetics* 9: 2471-2478.

Please replace paragraph [0013] with the following paragraph:

[0013] In one embodiment, the method of determining the presence of a Mucolipidosis IV mutant sequence in a nucleic acid sample, comprises in any order contacting the nucleic acid with a) a first oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 5124-5524 of the MCOLN1 gene **(SEQ ID NO: 8)**; b) a second oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 5541-**[[5491]]** **5941** of the MCOLN1 gene **(SEQ ID NO: 8)**; and c) an oligonucleotide probe that comprises a sequence complementary to a 13-30 bp segment of DNA that includes position 5534 ~~**between**~~ of the MCOLN1 gene **(SEQ ID NO: 8)**, wherein the probe is labeled with a detectable label that comprises a donor fluorophore and a quencher

moiety, wherein the quencher moiety is optionally an acceptor fluorophore. Once combined, amplification is conducted by temperature cycling and monitoring the accumulation of amplified nucleic in real time by detecting an increase in donor fluorophore fluorescence or a decrease in acceptor fluorophore fluorescence which indicates the presence of the Mucolipidosis IV mutant sequence in the nucleic acid.

Please replace paragraph [0014] with the following paragraph:

[0014] In another embodiment, the method of determining the presence of a Mucolipidosis IV mutant sequence in a nucleic acid, comprises in any order the steps of contacting the nucleic acid sample with; a) a first oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 100-500 of the MCOLN1 gene **(SEQ ID NO: 8)**, b) a second oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 6956-7356 of the MCOLN1 gene **(SEQ ID NO: 8)**, and c) an oligonucleotide probe that comprises a sequence complementary to a 13-30 bp segment of a fragment that is amplified using the first and second oligonucleotide primers, wherein the probe is labeled with a detectable label that comprises a donor fluorophore and a quencher moiety, wherein the quencher moiety is optionally an acceptor fluorophore. Once combined, amplification is conducted by temperature cycling and monitoring the accumulation of amplified nucleic in real time by detecting an increase in donor fluorophore fluorescence or a decrease in acceptor fluorophore fluorescence which indicates the presence of the Mucolipidosis IV mutant sequence in the nucleic acid.

Please replace paragraph [0015] with the following paragraph:

[0015] In yet another embodiment, the method of determining the presence of a Mucolipidosis IV mutant sequence in a nucleic acid, comprises in any order the steps of contacting the nucleic acid sample with; a) a first oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 100-500 of the MCOLN1 gene **(SEQ ID NO: 8)**; b) a second oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 6956-7356 of the MCOLN1

gene **(SEQ ID NO: 8)**, c) a first oligonucleotide probe that comprises a sequence complementary to a 13-30 bp segment of a fragment that is amplified using the first and second oligonucleotide primers, wherein the first probe is labeled with a first detectable label comprising a donor fluorophore and a quencher moiety wherein the quencher moiety is optionally an acceptor fluorophore; d) a third oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 100-500 of the MCOLN1 gene **(SEQ ID NO: 8)**, e) a fourth oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 6956-7356 of the MCOLN1 gene **(SEQ ID NO: 8)**, and f) a second oligonucleotide probe that comprises a sequence complementary to a 13-30 bp segment of a fragment that is amplified using the third and fourth primers, wherein the second probe is labeled with a second detectable label comprising a donor fluorophore and a quencher moiety, wherein the quencher moiety is optionally an acceptor fluorophore, and wherein the second detectable label is distinguishable from the first detectable label. Once combined, amplification is conducted by temperature cycling and monitoring the accumulation of amplified nucleic in real time by detecting an increase in donor fluorophore fluorescence or an increase or decrease in acceptor fluorophore fluorescence which indicates the presence of one or both of the Mucolipidosis IV mutant sequences in the nucleic acid.

Please replace paragraph [0022] with the following paragraph:

[0022] The present invention provides specific primers and probes that aid in the detection of ML IV mutant alleles or a wildtype allele. Primers are used to amplify one or more segments of the MCOLN1 gene. Probes are designed to detect an A>G transition (IVS 3-2 A>G), which causes a splice site mutation in the acceptor site of intron 3 is found in 72% of Ashkenazi ML IV alleles, and a 6.4 kb deletion that includes exons 1 to 7 of the MCOLN1 gene (GenBank accession no. AF287270) **(SEQ ID NO: 8)**. The primers and probes may be used in real time PCR. Simultaneous detection of two mutant alleles or two mutant alleles and a wildtype allele is made possible in real time PCR by labeling the probes with differentially detectable labels which comprise a donor fluorophore and a quencher moiety. The quencher moiety may optionally be an acceptor fluorophore. The separation of the donor fluorophore from the

quencher resulting from a conformational change in the probe upon hybridization (e.g., molecular beacon probe) or from degradation of the probe by a 5' nuclease activity of a DNA polymerase or reverse transcriptase in the PCR reaction (e.g., TaqMan® probe) reflects binding to amplified target and may be optically detected by an increase in emission of the donor fluorophore or by a decrease in emission of the acceptor fluorophore (it this feature is present in the quencher).

Please replace paragraph [0035] with the following paragraph:

[0035] Exemplary primers for amplifying segments of the MCOLN1 gene are given in Table 1. Additional IVS forward primers include those that hybridize within a 400 bp stretch of DNA from position 5124 to 5524 (accession # AF287270). Additional IVS reverse primers include those that hybridize within a 400 bp stretch of DNA from position **[[5141]]** **5541** to 5941 (accession # AF287270).

Please replace paragraph [0036] with the following paragraph:

[0036] Additional PRI forward primers include those that hybridize within a 400 bp stretch of DNA from position 100 to 500 (accession # AF287270). Additional PRI reverse primers include those that hybridize within a 400 bp stretch of DNA from position 6956 to 7356 (accession # AF287270).

Please replace paragraph [0038] with the following paragraph:

[0038] The term "detectable label" as used herein refers to a composition or moiety that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, radiochemical, or chemical means such as fluorescence, chemifluoresence, or chemiluminescence, or any other appropriate means. Preferred detectable labels are fluorescent dye molecules, or fluorophores, such fluorescein, phycoerythrin, CY3, CY5, allophycocyanine, Texas Red, peridenin chlorophyll, cyanine, FAM, JOE, TAMRA, TET, VIC. These examples are not meant to be limiting. Methods and compositions for detectably labeling molecules, such as oligonucleotides, PNA-DNA hybrids, etc. are well known in the art. See, e.g., U.S. Patents

6,316,230; 6,297,016; 6,316,610; 6,060,240; 6,150,107; and ~~6,028,290~~ **6,028,190**, each of which are hereby incorporated by reference in their entirety.

Please replace Table 1 with the following table:

Primer	Sequence	Genbank accession # AF287270
IVS-F	5'-AGC GGG CCG GAC TCA-3' (SEQ ID NO. 1)	~~5494~~ **5495**-5509
IVS-R	5'-TAA CCA CCA TCG GAT CAA TGT C-3' (SEQ ID NO. 2)	~~5671~~ **5677**-5698
PRI F1	**[[:]]**5'-CTT GCT CTG TTG CCC AGG CT -3' (SEQ ID NO. 3)	441-460
PRI R2	5'-CTC ACC GTG CTG GAA GAC ACT -3' (SEQ ID NO. 4)	7017-7037

Please replace paragraph [0050] with the following paragraph:

[0050] Exemplary probes for detecting amplified segments of the MCOLN1 gene as described herein are given in Table 2. The IVS wildtype or A>G transition probes may be extended or shortened on either end provided that the transition position (5534; Genbank accession no. AF287270) is not the end base of the probe. The probe for detecting the amplified fragment that results following deletion of 6.4 kb may hybridize anywhere within this amplified fragment. Using the outermost positions for the primers as described herein, the amplified fragment for the deletion mutant is about 800 bp assumes about and includes bases 100 to 500 followed by bases 6956 to 7356 (Genbank accession no. AF287270). A suitable probe in this case is one that consists essentially of from 13 to 30 nucleotides, preferably from 14 to 25 nucleotides of sequence anywhere in this approximately 800 **[[pb]] bp** of amplified DNA. Using the innermost positions for the primers as described herein and assuming a 40 base primer at each end, the amplified fragment for the deletion mutant is about 60 bp and includes bases 480 to

500 followed by bases 6956 to 6976 (Genbank accession no. AF287270). A suitable probe for this amplified fragment is one that consists essentially of from 13 to 30 nucleotides, preferably from 14 to 25 nucleotides of sequence anywhere in this approximately 60 **[[pb]]** **bp** of amplified DNA.

Please replace Table 2 with the following table:

ML IV sequence target	Probe Name	Sequence
IVS Wildtype	IVS WT	5'- TCTG CCC ACA GTA CCT -3' (SEQ ID NO: 5) (Genbank nt positions 5525-**[[5539]]** **5540**; accession # AF287270). The 5' end is labeled with VIC.
IVS A>G transition (IVS 3-2 A>G)	IVS MUT	5'- CTGC CCA CGG TAC CT -3' (SEQ ID NO: 6) (Genbank nt positions 5526-5540; accession # AF287270). The 5' end is labeled with FAM.
6.4 Kb deletion of MCOLN1 including exons 1-7.	DEL	5'- AGACC CAG GCC CAC AT- 3' (SEQ ID NO: 7) (Genbank nt positions 6982-6997; accession # AF287270). The 5' end is labeled with TET.

Please replace paragraph [0051] with the following paragraph:

[0051] In a preferred embodiment, real time PCR is performed using TaqMan® probes in combination with a suitable amplification/analyzer such as the ABI Prism 7900HT Sequence Detection System. The ABI PRISM® 7900HT Sequence Detection System is a high-throughput real-time PCR system that detects and quantitates nucleic acid sequences. Briefly,

TaqMan[[™]]® MGB probes specific for each allele are included in the PCR assay. These probes contain a reporter dye at the 5' end and a quencher dye at the 3' end. In addition, the minor-groove binding (MGB) component at the 3' end of the probe stabilizes the specific hybridization of a ~~TagMan™~~ **TaqMan®** probe to its DNA target and thus enhances the specificity of the assay. Each allele specific probe is conjugated with a different fluorescent reporter dye. During PCR, the fluorescently labeled probes bind specifically to their respective target sequences; the 5' nuclease activity of Taq polymerase cleaves the reporter dye from the probe and a fluorescent signal is generated. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR. A mismatch between probe and target greatly reduces the efficiency of probe hybridization and cleavage. The ABI Prism 7900HT Sequence detection System measures the increase in fluorescence during PCR thermal cycling, providing "real time" detection of PCR product accumulation.

Please replace paragraph [0055] with the following paragraph:

[0055] General sample volumes: **[[Whole]]** **whole** blood (5 ml per adult, 2 ml per child); amniotic **[[Fluid]]** **fluid** (10-15 ml); cultured cells (two T-25 culture flasks with 80-100% confluent growth); extracted DNA (100 ng or more in TE or sterile water). and chorionic **[[Villi]]** **villi** (10-20 mg in a sterile container with 2-3 mL of sterile saline or tissue culture medium). Whole Blood may be collected preferably a lavender-top (EDTA) tube or yellow-top (ACD) tube. Green-top (Sodium Heparin) tubes may also be used but are not preferred.

Please replace paragraph [0065] with the following paragraph:

[0065] Individual amplifications were prepared in a volume of 50 **[[☒]]** µl, which was added to 96 well microtiter plates. Each amplification volume contained 4 **[[☒]]** µl of the DNA sample (generally 80-160 ng of DNA) and 46 **[[☒]]** µl of working PCR master mix. Working PCR master mix comprised 25 µl of TaqMan 2x Universal Master Mix (Applied Biosystems, #4304437) and 21 **[[☒]]** µl of Mucolipidosis IV primer/probe mix. Mucolipidosis IV

primer/probe mix was prepared using 100 [[☒]] μM stock primers: IVS-F (SEQ ID NO: 1); IVS-R (SEQ ID NO:2); PRI-F1 (SEQ ID NO: 3); and PRI-R2 (SEQ ID NO: 4); 100 [[☒]] μM TaqMan probes: (Cat #s: 4316034, 4316033, 4316032, from Applied BioSystems. The fluorescent label is specified when entering the sequences); IVS-WT probe (SEQ ID NO: 5); labeled with VIC; IVS-MUT probe (SEQ ID NO: 6) labeled with FAM; and DEL probe (SEQ ID NO: 7) labeled with TET; 25 mM $MgCl_2$ (Qiagen #203205); 5X Q solution (Qiagen #203205); 100X BSA (NEB B90015); and molecular biology grade water (Bio Whittaker #16-001Y). Mucolipidosis IV primer/probe mix was prepared from these reagents as indicated in Table 3.

Please replace Table 3 with the following table:

Reagent	Final Conc.	Per rxn. (μL)	Cocktail (μL) x100
Nuclease-free dH_2O	NA	9.4	940
IVS-F Primer (100 [[☒]] μM)	0.9 μM	0.45	45
IVS-R Primer (100 [[☒]] μM)	0.9 μM	0.45	45
PRI F1 Primer (100 [[☒]] μM)	0.9 μM	0.45	45
PRI R2 Primer (100 [[☒]] μM)	0.9 μM	0.45	45
25 mM Mg	0.5 μM	1	100
5X Q Solution	4X	2.5	250
100X BSA	2X	1	100
IVS WT probe (10 [[☒]] μM)	0.2 μM	1	100
IVS MUT probe (10 [[☒]] μM)	0.36 μM	1.8	180
DelProbe1 (10 [[☒]] μM)	0.5 μM	2.5	250
Total		**21**	**2100**